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**14. ABSTRACT**
The goal of this proposal is to identify the proteins in human cells that are responsible for mutagenesis. Specific biochemical pathways are responsible for introducing mutation to the genome. Using drug(s) to inhibit one or more of these proteins and thereby prevent cancer is a novel and unique cancer prevention approach. Using a yeast deletion strain library, we developed and implemented high-throughput screens to identify genes involved in mutation. Using this approach, we identified all of the genes required for mutation induced by DNA damage resulting from ultraviolet light and methylmethane sulfonate. Most notably, the screens identified a novel pathway of induced mutation involving regulation of nucleotide substrates for a replicative DNA polymerase, in addition to identifying the known pathways of induced mutation involving the error prone translesion polymerases. Future work will involve further characterization of the novel mutation pathway as well as additional screens using different damage conditions and selections.

**15. SUBJECT TERMS**
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**Introduction**
The goal of our proposal was to identify the proteins in human cells that control the introduction of mutations into the genome using genome wide screens for mutants with a mutationless phenotype in the model eukaryote *S. cerevisiae.* The identification of human genes through their counterparts in yeast has been used in the past to identify many disease-related genes. The identified human proteins could serve as drug targets for the inhibition of mutation, thereby providing a novel approach to cancer prevention and treatment. This is our final report summarizing our findings between 15 MAY 2004 and 14 MAY 2007

**Body**
The overall goal of our proposal was to identify genes that are involved in introducing mutations into the human genome through the completion of three tasks:

*Task 1: Screening the yeast genome for candidate genes of the mutagenesis pathway*
*Task 2: Identification of bona fide yeast mutagenesis genes.*
*Task 3: Identification of mutagenesis genes in humans*

We screened a library of yeast deletion strains representing all of the non-essential yeast genes for a mutationless phenotype. While high-throughput screening of the genome was easily accomplished on schedule with the timeline of our original proposal, verification of the phenotype of the identified strains (Task 2) was laborious and required more effort than originally anticipated mainly due to problems associated with the library deletion strains, which were obtained commercially. We and others (*1*) have observed both aneuploidy and polyploidy among these deletion library strains. These chromosomal abnormalities led to false positives (see below).

Analysis of all the screening data (described in our interim reports) yielded over 250 putative mutationless genes. We determined quantitative spontaneous and damage induced mutation rates for all of these strains to evaluate their phenotype compared to wild type and the known mutationless strains (*i.e.* those lacking the *REV* genes). From these experiments, 135 strains were found to have UV-induced mutation rates that were less that 70% of the wild-type rate.

As we reported last year, we next reconstructed these deletion mutants in a different yeast strain (RDKY3615) to allow us to a) perform two additional mutation assays and b) rule out anomalies associated with the deletion library strains. We found that 151 of the 267 identified strains had an apparent UV-induced $Can^r$ forward mutation frequency more than 1.5-fold lower than wild type, with similar trends observed for the spontaneous mutation frequencies. The mutation frequencies determined for the remaining 116 strains were all within experimental error of wild type.

We next examined whether the apparent decreases in mutation frequencies observed in the isolated library strains resulted from disruption of the specific open reading frame. Deletion mutants corresponding to the 151 library strains were re-constructed in strain RDKY3615. Analysis of $Can^r$ mutation in these strains identified 20 with a UV-induced mutation frequency at least 2-fold lower than wild type. Included among these 20 strains were *RAD6*, *RAD5*, and *RAD18*, whose role in spontaneous and damage-induced mutation is well known; these genes were not characterized further. For the 17 remaining genes, we next characterized UV-induced reversion of the +4 *lys2ΔBgl* and +1 *hom3-10* frameshift alleles, as well as forward mutation of genes conferring resistance to the toxic cytosine analog fluorocytosine (5-FC) (Tables 1 and 2). These constitute both gain- and loss-of-function mutation assays, and together they differentiate between strains that are truly defective for

induced mutation and those that are only apparently defective due to artifacts, such as polyploidy. Two clearly different phenotypes were observed: (1) significantly diminished mutation frequencies in the forward mutation assays (loss of function loci), but elevated frequencies in the reversion assays (gain of function loci); and (2) reduced mutation frequencies in all assays. Genes in the former group were all found to be involved in the regulation of chromosome number, not in induced mutation. Analysis of the DNA content of the mutants in phenotypic group I revealed that they all have at least twice as much DNA per cell, relative to stationary phase wild-type cells. In addition, these mutants are morphologically abnormal, exhibiting divided, but not separated cells. These mutants were falsely identified by the screen and while scientifically interesting, they are irrelevant to the proposed work and will not be discussed here.

Of the genes whose deletion results in reduced mutation in both gain- and loss-of-function assays, two were not further characterized. The decrease in mutation frequency observed upon deletion of *KAP123*, which encodes a protein involved in nuclear import of ribosomal proteins and histones (*2*), was significantly smaller than that for the other strains in all of the mutation assays. In the case of *VPS3*, which encodes a protein involved in sorting and processing of soluble vacuolar proteins (*3*), wild type mutation rates were observed when more time was allowed between UV irradiation and Can$^r$ selection, suggesting that the apparently reduced rates were an artifact associated with phenotypic lag. This recovery of induced mutation was not observed for any of the other deletions strains identified.

Five of the identified genes, *REV1*, *REV3*, *REV7*, *POL32*, and *SRS2*, are already known to be involved in mutation. Consistent with previous reports, *rev1*Δ, *rev3*Δ, and *rev7*Δ show a reduction in both spontaneous and UV-induced mutation at *CAN1* (Tables 1 and 2). In addition, we show that *REV1*, *REV3*, and *REV7* also play an important role in induced frameshifts (*lys2*Δ*Bgl* and *hom3-10* assays) as well as in spontaneous and UV-induced mutations that confer resistance to 5-FC (Tables 1 and 2). The remaining two genes, *FYV6* and *RNR4*, are much less well characterized, but also appear to play a general role in induced mutation.

**Table 1.** Spontaneous mutation frequencies[a]

| Genotype | Can$^r$ $\times 10^{-8}$ | 5-FC$^r$ $\times 10^{-8}$ |
|---|---|---|
| Wt | 21.6 | 19.7 |
| *vps3*Δ | 16.7 | 52.0 |
| *rev1*Δ | 9.97 | 1.70 |
| *rev3*Δ | 7.89 | 2.40 |
| *rev7*Δ | 8.07 | 4.60 |
| *pol32*Δ | 25.2 | 8.70 |
| *rnr4*Δ | 14.3 | 5.50 |
| *srs2*Δ | 9.71 | 15.9 |
| *kap123*Δ | 23.3 | 9.98 |
| *fyv6*Δ | 16.6 | 40.7 |
| WT[b] | 25.9 | nd |
| *rnr4*Δ[b] | 26.8 | nd |
| *pol3-1*[b] | 1130 | nd |
| *pol3-1 rnr4*Δ[b] | 399 | nd |
| *pol2-4*[b] | 128 | nd |
| *pol2-4 rnr4*Δ[b] | 40.9 | nd |

[a] Spontaneous mutation frequencies were determined by fluctuation analysis using the method of the median from 9 parallel cultures. Values reported are an average of 2-3 determinations.
[b] rates were determined in strain S18-1 background
nd not determined

**Table 2.** UV-induced mutation frequencies[a]

| Genotype | Can$^r$ $\times 10^{-6}$ | 5-FC$^r$ $\times 10^{-6}$ | *lys2*Δ*Bgl* $\times 10^{-7}$ | *hom3-10* $\times 10^{-7}$ |
|---|---|---|---|---|
| wt | 151 ± 21 | 222 ± 30 | 18.1 ± 2.3 | 10.0 ± 1.8 |
| *vps3*Δ | 1.30 ± 1.3 | 114 ± 22 | 12.3 ± 2.3 | 9.72 ± 4.1 |
| *rev1*Δ | 5.15 ± 1.0 | 35.4 ± 11 | 0.93 ± 0.5 | 2.54 ± 1.2 |
| *rev3*Δ | 5.79 ± 1.3 | 39.7 ± 5.8 | 1.36 ± 1.0 | 1.12 ± 0.1 |
| *rev7*Δ | 6.90 ± 0.6 | 49.6 ± 33 | 0.72 ± 0.4 | 2.50 ± 1.6 |
| *pol32*Δ | 19.4 ± 5.3 | 34.9 ± 12 | 1.15 ± 0.6 | 5.62 ± 2.0 |
| *rnr4*Δ | 29.8 ± 6.4 | 82.0 ± 16 | 5.34 ± 2.7 | 8.92 ± 1.6 |
| *srs2*Δ | 67.4 ± 34 | 28.2 ± 5.9 | 9.31 ± 3.5 | 7.72 ± 5.4 |
| *kap123*Δ | 72.1 ± 11 | 94.3 ± 22 | 19.4 ± 1.6 | 12.9 ± 1.3 |
| *fyv6*Δ | 75.9 ±12 | 180 ± 5.0 | 12.1 ± 2.3 | 6.89 ± 1.4 |

[a] UV-induced mutation frequencies were determined using a minimum of 3 parallel cultures.

*FYV6* and *RNR4* thus appeared to be *bona fide,* novel mutatgenesis genes. To better understand their role in induced mutation, we first determined whether they function in the same pathway(s) as the known TLS polymerases; these experiments were proposed in our original submission. As we were nearing the end of our funding period, we chose not to pursue yeast two hybrid studies to identify other proteins acting in concert with either Rnr4 or Fyv6 and instead we performed other more facile, less costly, but equally insightful experiments designed specifically for these genes to characterize their role in mutation (see below).

Genetic epistasis analysis using UV induced mutation of *CAN1* suggests that Fyv6 and Polζ function independently, as an additive decrease in mutation frequency was observed with deletion of both *FYV6* and *REV3* (Figure 1*A*). Analysis of the Can$^r$ mutation spectra (Table 3) showed that mutations induced in *fyv6*Δ cells are clearly distinct from those induced in *rev3*Δ cells (*4, 5*), but similar to those induced in wild type cells (*5*). We also examined EMS-induced mutation of *CAN1*. Unlike, deletion of *REV3*, deletion of *FYV6* results in a small but significant reduction in EMS-induced mutagenesis (Figure 2*A*).

It has been speculated that Fyv6 plays a role in the regulation of non-homologous end joining (NHEJ) (*6*). However, we found that *fyv6*Δ is not deficient for NHEJ using a plasmid re-joining assay. We also examined whether loss of NHEJ leads to reduced UV-induced Can$^r$. *yku70*Δ, *lif1*Δ, *dnl4*Δ and *mre11*Δ strains, all of which are known to be defective in NHEJ, showed wild type mutation rates (as expected since they were not isolated in the screen, data not shown). Thus we conclude that any NHEJ activity of Fyv6 does not contribute to its role in induced mutation.

**Table 3.** UV-induced Can$^r$ mutation spectra

| Genotype | Mutation | Frequency (%) |
|---|---|---|
| wt | T→C or G→A | 10/20 (50) |
| | T→A or A→T | 4/20 (20) |
| | C→A or G→T | 2/20 (10) |
| | frameshift | 3/20 (15) |
| | complex | 1/20 (5) |
| *rev3*Δ | T→C or G→A | 17/20 (85) |
| | G→C | 1/20 (5) |
| | frameshift | 2/20 (10) |
| *rnr4*Δ | T→C or G→A | 9/30 (30) |
| | T→A or A→T | 9/30 (30) |
| | C→A or G→T | 3/30 (10) |
| | G→C | 1/30 (3.3) |
| | frameshift | 6/30 (20) |
| | complex | 2/30 (6.7) |
| *fyv6*Δ | T→C or G→A | 8/20 (40) |
| | T→A or A→T | 5/20 (25) |
| | frameshift | 4/20 (20) |
| | complex | 3/20 (15) |

We also examined whether Rnr4 acts in the same or related pathways as the conventional TLS polymerases. As with *FYV6*, simultaneous disruption of *RNR4* and *REV1* or *REV3* results in an additive decrease in mutation frequency relative to the corresponding single mutants (Figure 1*B* and 1*C*). Deletion of *RAD30* has no effect in either the wild type or the *rnr4*Δ strain (Figure 1*D*). Also as was observed with *fyv6*D, the UV-induced Can$^r$ mutation spectrum of *rnr4*Δ is clearly distinct from *rev3*Δ, but similar to wild type cells (Table 3). This demonstrates that Rnr4 does not function in mutagenic PRR. Furthermore, EMS induces no Can$^r$ mutation in *rnr4*Δ cells (Figure 2*A*). Finally, we determined that *FYV6* and *RNR4* are epistatic for mutation at *CAN1* (Figure 1*E*). **These data indicate that *RNR4* and *FYV6* function in the same pathway and that this pathway is independent from the pathway involving Rev1, Pol ζ and/or Polη.**

Because the effects were larger with *RNR4*, we next examined whether it cooperates with a replicative polymerase to induce mutation. The replicative polymerases Polδ and Polε both have 3' to 5' exonuclease proofreading activity that may contribute to replication fidelity. We characterized the spontaneous and UV-induced Can$^r$ mutation rates of *pol3-1* and *pol2-4* strains, where the exonuclease activities of Pol δ and Polε, respectively, have been disrupted (*7, 8*). Disabling Polε proofreading results in a modest increase in spontaneous mutation (Table 1) that is partially dependent on *RNR4*, and it has no effect on UV- or EMS-induced mutation in either the wild type or *rnr4*Δ strains (Figure 1*G* and 2*B*). In contrast, disabling Polε proofreading results in a dramatic increase in both spontaneous and UV- or EMS-induced mutation. Remarkably, this increase in mutation is entirely dependent on *RNR4* (Figure 1*F* and 2*B*). Thus, *RNR4* mediated induced mutation appears to act specifically via Polδ.

Lastly, we determined the relationship between Rnr4 activity and two major pathways that mediate repair of UV-induced DNA damage, nucleotide excision repair (NER) and PRR. To determine whether Rnr4 is involved in NER, the primary repair mechanism responsible for removing UV-lesions, we examined the UV sensitivity of a *rad1*Δ *rnr4*Δ mutant. We found that the *rnr4*Δ *rad1*Δ double mutant was not significantly more sensitive to UV-light than the *rad1*Δ mutant. In addition, the *rnr4*Δ *rad1*Δ mutant had lower UV-induced mutation frequencies than the *rad1*Δ mutant (Figure 1*H*), implying that in the absence of NER, Rnr4 is still required for the efficient induction of mutation. To determine whether Rnr4 is involved in error-free PRR (a role in error-prone PRR is excluded by the experiments with *REV3*, described above) we examined the DNA damage sensitivity of a *rad18*Δ *rnr4*Δ mutant. We found that a *rnr4*Δ *rad18*Δ mutant was synergistically more sensitive to UV, MMS, or EMS exposure than either single mutant (Figure 2*C*), suggesting that Rnr4 functions to help tolerate DNA damage that otherwise requires the error-free branch of PRR.

We next demonstrated that deletion of *RNR4* reduces and imbalances intracellular nucleotide pools. These experiments are somewhat beyond the scope of our original proposal, but have already provided us with very interesting results. *rnr4* mutants are known to progress more slowly through S-phase (*9, 10*). To investigate whether *rnr4*Δ mutant cells have lower levels of dNTPs than wild type cells, we are analyzing the amount of dNTPs that are present in wild type and *rnr4*Δ strains, both in undamaged cells and in cells damaged with UV, NQO, or EMS. Although not a potent inducer, we find that UV irradiation increases the level of dNTPs in wild type, but not *rnr4*Δ cells. Analysis of this data is still in progress.

Hydroxyurea (HU) is a small molecule chemotherapeutic that is thought to slow tumor growth by inhibiting RNR and thus reducing dNTP concentrations (*11*). To confirm that increases in nucleotide pools can result in reduced mutation, and to determine whether induced mutation may be inhibited by a small molecule, we examined the effects of HU. HU significantly inhibits UV-induced mutation in a dose-dependent manner (Figure 2*D*). Remarkably, at 100 mM HU, the Can$^r$ mutation frequency per viable cell after 67 J/m$^2$ of UV light (7% survival) is reduced 100-fold to spontaneous levels. A similar reduction in UV-induced mutation frequency was observed with *lys2*Δ*Bgl* (Figure 2*D*), implying that HU inhibits the formation of both point mutations and frameshift mutations. In addition, HU also potently inhibits EMS-induced mutation (Figure 2), and its inhibition of UV-mutation is independent of *RAD30*, *POL30*, and *REV3* (Table 4), further suggesting that HU specifically inhibits the same pathway that is mediated by Rnr4.

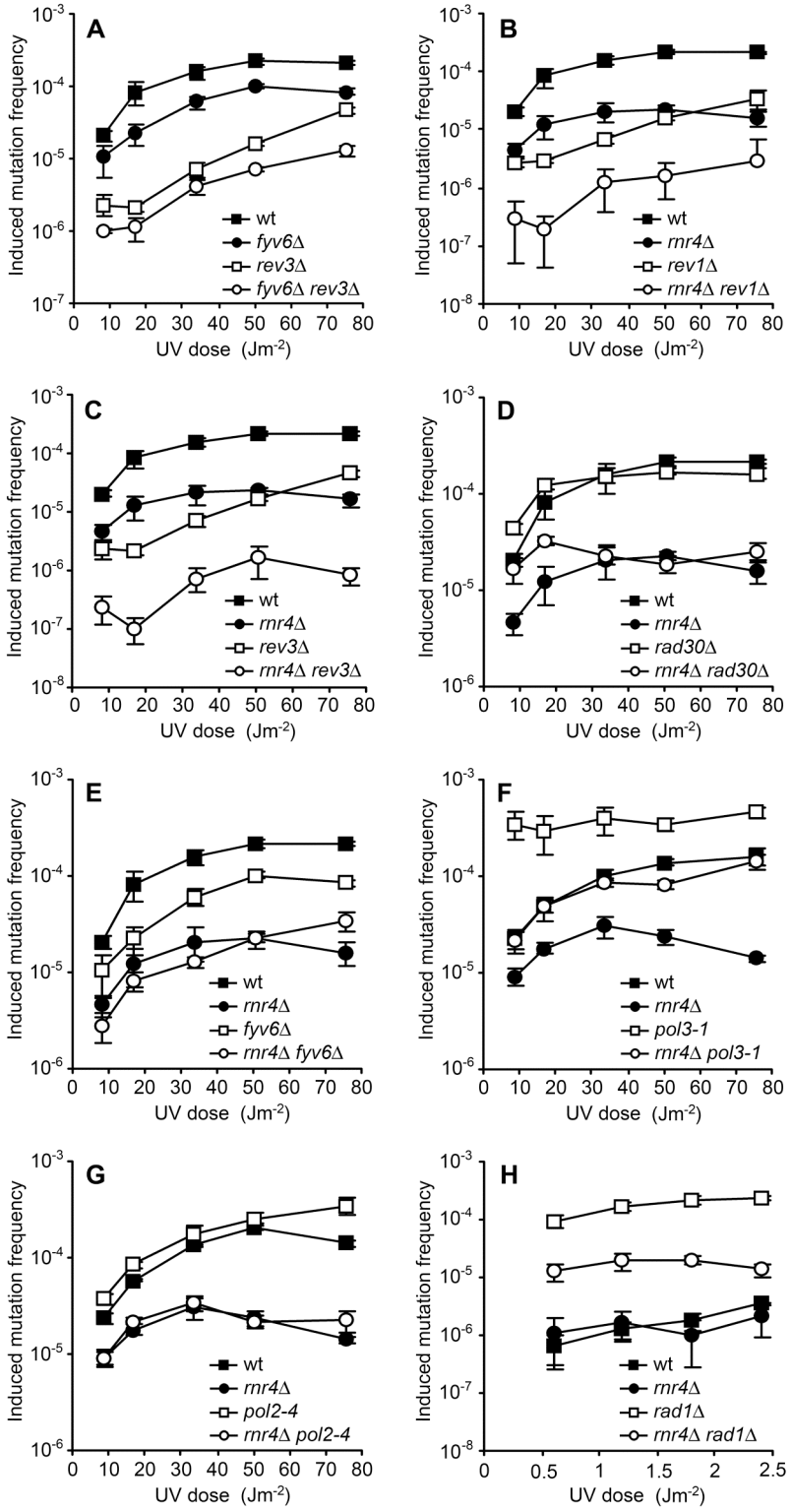


**Figure 1**. *RNR4* and *FYV6* function in a pathway that is distinct from the TLS polymerases. (*A*-*H*) UV-induced mutation frequencies were determined as follows: cells from saturated cultures (grown for 24 h) were harvested by centrifugation, washed and resuspended in saline, plated on SC-Arg plates and irradiated with varying doses of UV light. Cells were allowed to recover for 6 h at 30 °C after which they were washed off the SC-Arg plates and re-plated onto media containing canavanine to identify Can$^r$ mutants and onto SC-Arg to determine the number of viable cells. All plates were incubated at 30 °C for three days after which the number of colonies was counted. Induced mutation frequencies were determined by dividing the number of Can$^r$ mutants observed by the number of viable cells and subtracting the contribution of the pre-existing, *i.e.* spontaneous, mutations. Induced frequencies were determined from 3-8 independent cultures.

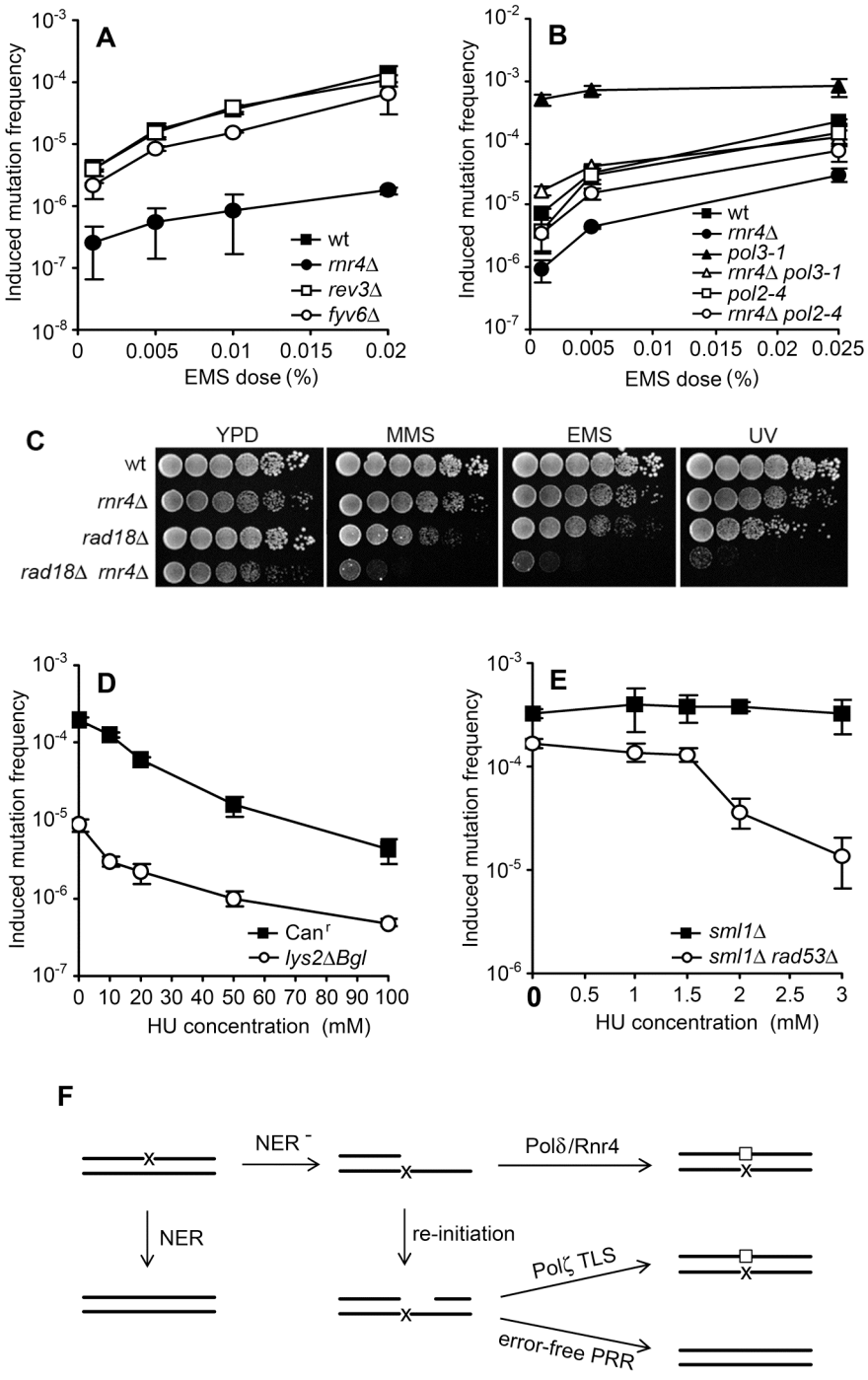


**Figure 2.** Effect of *RNR4* and HU on repair and mutagenesis. (*A,B*) EMS-induced mutation frequencies were determined as for UV-induced mutation frequencies in Figure 1 with the exception that EMS was present in SC-Arg plates instead of treating the plates with UV light. (*C*) *RNR4* and *RAD18* are strongly synergistic to DNA damaging agents. The sensitivity of the mutants was characterized by growing cells to mid-log phase, normalizing by cell density, and serially diluting in 5-fold increments. Five-microliter drops were plated on YPD, YPD subsequently irradiated with 17J/m$^2$ of UV light, and YPD containing 0.0002% MMS or 0.005% EMS. Plates were grown for 3 days at 30°C, and then photographed. (*D,E*) Anti-mutagenic effect of HU. To determine the number of mutants, cells from saturated cultures were plated on SC-Arg medium containing both canavanine and different concentrations of HU and immediately irradiated with 50 J/m$^2$ of UV light. To determine the number of viable cells, cells were plated on SC-Arg medium containing different concentrations of HU and immediately irradiated with 50 J/m$^2$ of UV light. Induced mutation frequencies were determined by dividing the number of Can$^r$ mutants observed by the number of viable cells and subtracting the contribution of spontaneous mutations. (*F*) Model of UV damage repair/bypass.

At high concentrations (~200 mM), HU induces S-phase arrest of *S. cerevisiae* (*12*). To determine whether the HU-mediated inhibition of mutation, which occurs at concentrations as low as 10 mM, results from a prolonged S-phase (perhaps allowing more time to repair UV lesions), the UV-induced mutation frequency with or without HU was determined for a *rad53*Δ mutant, which is unable to delay S phase progression following genotoxic stress. (Examining the same effect with the *rnr4*Δ mutant was not possible, since the *rnr4*Δ *rad53*Δ double mutant is inviable (*9*).) With or without the activity of Rad53, HU induces a profound reduction in mutation, suggesting that a checkpoint-mediated delay in S-phase is not required (Figure 2*E*).

**Table 4.** Genetic dependence of the anti-mutagenic effect of HU

| Genotype | Mutation Frequency[a] 0 mM ($\times 10^{-6}$) | 25 mM ($\times 10^{-6}$) | 50 mM ($\times 10^{-6}$) |
|---|---|---|---|
| wt | 221 ± 22 (1.00)[b] | 36.2 ± 11 (0.17) | 11.1 ± 1.1 (0.06) |
| *rev3*Δ | 2.89 ± 0.9 (1.00) | 0.47 ± 0.3 (0.16) | 0.28 ± 0.3 (0.10) |
| *rad30*Δ | 68.3 ± 18 (1.00) | 5.14 ± 1.7 (0.08) | 3.41 ± 1.0 (0.05) |
| *pol32*Δ | 4.16 ± 0.7 (1.00) | 0.47 ± 0.6 (0.11) | slow growth[c] |
| *sml1*Δ | 331 ± 76 (1.00) | 131 ± 25 (0.42) | 58.5 ± 11 (0.16) |

[a] Induced mutation frequency at the HU dose indicated following treatment with 50 J/m$^2$ UV light.
[b] Figures in parenthesis represent the relative rate for each strain. Induced mutation frequencies at 25 and 50 mM HU were normalized by the mutation frequency at 0 mM HU for each strain.
[c] Strain showed a significant sensitivity to 50 mM HU; induced mutation frequencies were not determined.

To further support the suggestion that the antimutagenic effect of HU is mediated through reduced dNTP levels, we examined the effect of HU in the absence of *SML1*. Sml1 is a protein inhibitor of RNR, and nucleotide levels are known to be elevated in *sml1*Δ strains (*13, 14*). The anti-mutagenic effect of HU was clearly suppressed in the *sml1*Δ strain, consistent with the effect being mediated by a reduction in nucleotide pools (Table 4).

The final objective of our original proposal was to identify the human homologs of the identified yeast genes. Rnr4 is one of four subunits comprising a ribonucleotide reductase; it shares about ~45% identity with a human ribonucleotide reductase subunit (M2), while Fyv6 does not appear to have a human homolog. However, the critical finding from this work is that dNTP levels influences the mutagenic outcome of DNA damage and mutation may result from translesion synthesis catalyzed by a replicative polymerase. This mechanism is very likely a component of the damage response in human cells and we intend to seek funding for the investigation of this hypothesis.

A model consistent with all of the data is presented in Figure 2*F*. In the case of UV-damage, the majority of photo-lesions are repaired by NER, but unrepaired photo-lesions, or lesions induced by other types of mutagens (*i.e.* MMS or EMS) that are not recognized by NER, cause replication forks to stall during S-phase and also induce the intra-S checkpoint response. As part of this response, Rnr4 is upregulated and the resulting increase in dNTPs facilitates TLS by Polδ and continued synthesis of contiguous DNA, but at the expense of an increased mutation rate (which is also facilitated by rendering Polδ exonuclease deficient). If TLS does not occur, genome replication is completed by the re-initiation of synthesis downstream of the blocking lesion (possibly in a manner similar to that recently observed in *E. coli* (*15*)), with the accumulation single-stranded DNA gaps. The gaps are then repaired either by error-free or mutagenic PRR.

The Rnr4-mediated changes in dNTP levels appear to affect Polδ specifically. While any polymerase will be more prone to synthesize through damaged templates at higher dNTP concentrations, Polδ may have reduced fidelity and/or proofreading activity, relative to Polε (which is likely the main

replicative polymerase responsible for leading strand synthesis), that render it more capable of TLS. It is interesting to speculate that Polδ acts as a default TLS polymerase during bulk DNA replication. The absence of an effect on Rev1 and Polζ, which appear to act cooperatively, is likely due to the temporal separation of *RNR4* and *REV1* transcription (with the former induced by damage during DNA replication (*16*) and the latter induced only in late S-phase when the bulk of DNA replication is complete (*17*)). Indeed, this is consistent with the role of *REV1*, *REV3*, *REV7*, and *RAD30* in *post*-replication repair. This model is also consistent with recent studies in bacteria (*15*).

Both Rnr4/Polδ and mutagenic PRR appear to contribute to mutation induced by many types of DNA damage, including UV and MMS, but only the Rnr4/Polδ pathway appears to play an important role in EMS-induced mutagenesis. It seems likely that UV- and MMS-induced damage are more potent blocks of replication, and thus are more likely to require the specialized activities of the conventional TLS polymerases, while EMS induces damage that may be replicated through by Polδ, at least in the presence of elevated levels of dNTPs.

As we reported in 2006, our results represent a considerable amount of work and to our knowledge *is one of the most comprehensive surveys of mutation rates, both in terms of number of genes analyzed and number of different assays used*. Preparation of a manuscript describing the complete data set and analysis of the identified genes is nearly complete.

**Key Research Accomplishments**

- Completion of genome wide screen of *S. cerevisiae* deletion strain library for strains incapable of forward mutating the *CAN1* gene

- Completion of genome wide screen of *S. cerevisiae* deletion strain library for strains incapable of forward mutating the *LYS2* or *LYS5* genes

- Identification of *all* of the genes previously known to be involved in mutation (*REV1, REV3, REV7, POL32, SRS2*)

- Identification and verification of two novel genes involved in induced mutation, *RNR4* and *FYV6*

- Identification of a novel pathway of induced mutation in yeast and very likely in humans that is independent of the known *REV* pathway and that instead involves the replicative polymerase Polδ

- Identification of hydroxyurea as an inhibitor of induced mutation that presumably acts by inhibiting ribonucleotide reductase and thereby decreasing the intracellular concentration of nucleotides

- Identification of importance of nucleotide pools in induced mutation, a mechanism that is likely also important in human cells

**Reportable Outcomes**

- Manuscript describing the screen, confirmation of phenotypes, and characterization of identified genes is near completion for submission to *Proc. Natl. Acad. Sci. USA*

- Applied for and was awarded funding from the California Breast Cancer Research Program for one graduate student

• Applied for and was awarded funding from the Tobacco Related Disease Research Program for one graduate student

• Ph.D. granted to B.A. O'Neill, 2007

• Numerous presentations, including universities and symposia; most notably the Ambassador's presentation at the 2005 Epigenetics Gordon Conference

**Conclusion**

To identify proteins that are required for induced mutagenesis, we carried out a screen for non-essential genes in *S. cerevisiae* that when absent, render cells less able to mutate. The identified genes included all of the non-essential genes previously known to be involved in mutation, *REV1*, *REV3*, *REV7*, *POL32*, *SRS2*, *RAD5*, *RAD6* and *RAD18*, and two novel genes, *FYV6* and *RNR4*. Since all of the genes previously known to play a role in induced mutation were identified, we conclude that the screen identified all of the non-essential genes required for UV- and MMS-induced mutation, at least at the *CAN* locus.

Genetic and biochemical characterization of *FYV6* and *RNR4* revealed that they act together to induce mutations in response to damaged DNA via the replicative polymerase Polδ in a pathway that is independent of the TLS polymerases. We show that HU inhibits this process, and we propose that if similar mechanisms are conserved in human cells, the mutations induced during chemotherapy might be inhibited. The potential therapeutic utility of such an approach depends on the proportion of mutations mediated by the two mechanisms of induced mutation, which is currently under investigation. Whatever the result, the results of our research sponsored by this IDEA award suggest that there are a finite number of pathways, perhaps only two, that independently induce mutation, so HU combined with an inhibitor of Polζ might represent a therapeutic combination that efficiently eliminates all forms of chemotherapy-induced mutation. Studies directed towards reaching these goals promise to provide an unprecedented opportunity to understand genome instability in eukaryotic cells and also to intervene in mutation, and thus in the development and treatment of cancer.

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